REMARKS

Reconsideration and allowance of the subject application are respectfully requested.

In the October 15, 2003 Office Action, the Examiner has withdrawn all of the previous art rejections. However, the Examiner has made two new rejections citing two new references, and we offer the following comments to address these.

Claims 1-7 are now rejected under 35 U.S.C. §103(a) as obvious over Kurn et al. (US Patent 6,200,757) in view of Mian et al. (U.S. Patent 5,686,271). The Examiner has proffered that it would have been obvious to modify the method of cycle primer extension at low temperatures as taught by Kurn with the teachings of Mian to develop the claimed method for cycle primer extension because Mian states that it is beneficial to carry out duplex dissociation at a temperature near the Tm of the duplex, and the dissociation should occur at a temperature that does not destabilize the polymerase enzyme used for primer extension. The Examiner stated that Mian suggests using agents that lower the Tm, such as lower alkyl alcohols, urea, formamide and other hydrogen bond competitors. Thus, the Examiner reasons that an ordinary practitioner would have been motivated to modify Kurn's method by incorporating the parameters or limitations of Mian (e.g., adding glycerol and ethylene glycol), since inclusion of such limitations would improve specificity of polymerase mediated primer extension.

As described in Kurn's abstract, that invention relates to "a method for selectively extending an oligonucleotide primer along a specific target polynucleotide sequence in a mixture of polynucleotides." The method consists of providing "an oligonucleotide primer having a modification, and a binding substance for the modification wherein the binding substance binds to the oligonucleotide and prevents the extension of the oligonucleotide along the target polynucleotide sequence." Then the temperature... is "adjusted to a level sufficient to irreversibly denature the binding substance and permit the extension of the oligonucleotide primer along the specific target polynucleotide sequence." As described the background section at column 1, lines 55-57, the problem

sought to be solved by Kurn's invention was that previous amplification methods suffer from interference caused by random partial hybridization of primers used in such amplification to irrelevant DNA. Kurn describes how such premature hybridization and extension of an oligonucleotide primer is prevented by specific binding of the oligonucleotide primer by a thermally labile binding substance such as a protein. (Column 5, lines 31-34)

Kurn further states:

> The present invention enables assembly of all reagents required for amplification in one reaction mixture. . . . Since the binding substance is rendered irreversibly inactive at the elevated temperature, it does not interfere in later analysis of the amplification reaction. Accordingly, the present invention results in elimination of non-specific priming at low temperatures, which is a major contributor to the production of non-specific amplification. . . . Column 5, lines 52-63

> When all of the amplification reagents are mixed with the sample, extension of the primer along any template polynucleotide present in the mixture is inhibited because of the presence of the complex between the binding substance and the oligonucleotide primer. As the temperature is increased, the binding substance dissociates from the complex with primer. The oligonucleotide primer (if previously unbound) can then bind to the template polynucleotide sequence and under go chain extension. Column 6, line 28-36

> One such procedure is the amplification of a target polynucleotide sequence, e.g. an amplification carried out using thermal cycling. . . . Column 6, lines 48-52

> In the context of an amplification, chain extension usually involves temperature cycling, i.e., elevating the temperature of the reaction mixture to cause hybridized polynucleotide sequences to denature, cooling the reaction mixture to permit binding of an oligonucleotide primer to its respective target polynucleotide sequence, and repeating the above. However, target polynucleotide may be amplified without thermocycling. Column 7, lines 9-17

In summary, Kurn's patent teaches using an antibody to bind a modified segment of an oligonucleotide primer to prevent premature non-specific hybridization of the primer to irrelevant polynucleotides in the reaction mixture until a high temperature causes irreversible separation of the antibody from the primer during PCR temperature

cycling to ensure specific hybridization between the target oligonucleotide and its primer from the start of the PCR or primer extension. It does not consider the issue addressed by our invention, namely, the need for a low-temperature cycling system with which sequence-specific extension of primers of shorter than 30 base pairs can be achieved to generate useful amplification DNA products for sequencing and for further molecular analysis (among other issues).

The Examiner stated on page 3 of the Office Action, that "Kurn et al. teach a method of claim 1, for extending an oligonucleotide primer or a pair of primers using an enzymatic cycle primer extension (see column 6, lines 21-55, column 22, lines 44-67, column 23, lines 1-19) at temperatures between about 45°C and about 65°C and a melting temperature of about 70°C (see column 19, lines 41-45, column 23, lines 2-19)". However, this cannot be correct. As noted above, column 6, lines 28-36 and 48-52 describes how extension of the primer along any template polynucleotide present in the mixture is inhibited because of the presence of the complex between the binding substance and the oligonucleotide primer, since as the temperature is increased, the binding substance dissociates from the complex with primer. The oligonucleotide primer (if previously unbound) can then bind to the template polynucleotide sequence and undergo chain extension (citing as an example thermal cycling amplification). The patent at columns 22 and 23 states that "...each cycle includes heating the medium at about 90° C. to about 100° C for about 2 seconds to about 3 minutes....."(column 23, lines 5-6) and "... preferably, at about 90° C . to about 99° C. for about 10 seconds to about 2 minutes..." (column 23, lines 15-16). In other words, **Kurn et al. still relied on the traditional PCR using a melting temperature of about 95° C and a heat-resistant DNA polymerase, like Pfu DNA polymerase** (used in all of the Examples 1-5).

This is also borne out by the Examples, especially Example 1 at column 33, lines 48-52 ("The reaction mixture (total volume 25 μl) were subjected to thermal cycling as above. Thermocycling for the first PCR amplification reaction was as follows: 4 min. at 94° C; 35 cycles of 30 sec at 94° C, 1 min. at 64°C and 1 min. 72° C.); Example 2 at column 35, lines 26-29 ("A total of 40 cycles . . . were performed consisting of a 30 sec. denaturation step at 94° C, a 1 min reannealing step at 64° C and a 1 min extension step at

72° C, preceded by a 4 min. denaturation of genomic DNA at 95° C."); and Example 5 at column 39, lines 42-46 (". . . 4 min at 95° C,...40 cycles of 30 sec at 94° C...."). Examples 3 and 4 use the same conditions as Example 2. Thus, Kurn does not teach a method for enzymatic cycle primer extension at temperatures between about 45°C and about 65°C and a melting temperature of about 70°C—which temperature parameters are affirmatively recited and required in our claims 1-7.

Furthermore, although Kurn included rBST DNA polymerase from Epicentre Technologies (Column 14, line 34, also noted by the Examiner in the Office Action) as one of at least eleven potentially useful DNA polymerases, there is no teaching or description or hint of suggestion regarding the conditions under which a moderately thermostable DNA polymerase like *Bacillus stearothermophilus* can survive a high temperature of 94° C. Indeed, as the present inventors now know, without the protection of between 10% and 20% glycerol, ethylene glycol or a mixture of these, the moderately thermostable Bacillus DNA polymerases are readily inactivated at 70° C. Kurn simply did not address this or even consider it.

Someone having ordinary skill in this art could not have learned or inferred from Kurn that enzymatic cycle primer extension reaction can be carried out using *Bacillus stearothermophilus* at temperatures between about 45°C and about 65°C, and a melting temperature of about 70°C, repeatedly, so that the cycle reaction temperature fluctuates between the 70°C melting temperature and an annealing temperature of about 37°C, to produce a sequence-specific amplification product, irrespective of whether Kurn teaches the use of solution containing between about 10% and about 20% glycerol and/or ethylene glycol (which the Examiner noted it does not).

To make up for this last deficiency—the lack of teaching in Kurn of reaction solution containing between about 10% and about 20% glycerol and/or ethylene glycol—the Examiner cited the Mian patent U.S. 5,686,271. However, this patent relates to an entirely different method of performing oligonucleotide primer extension, referred to as magnetic cycle reaction (MCR). Its principle is described in column 8, line 55—column 9, line 7 as follows:

> The basic steps involved in the MCR process for amplifying a single-stranded target nucleic acid sequence are as follows: First, a solid phase or magnetic primer is incorporated into a nucleic acid strand that is complementary to the target nucleic acid sequence. This step yield a double stranded target nucleic acid that has one strand bound to either a solid phase or magnetic primer, respectively, the solid phase strand or the magnetic strand. Second, the target nucleic acid sequence and its complement are denatured. Third, a magnetic or solid phase primer, whichever was not incorporated into the complementary strand, is incorporated into a nucleic acid strand that is homologous to the target nucleic acid sequence, i.e. complementary to the solid phase or magnetic strand. These steps provide a double-stranded nucleic acid sequence that has strand bound to a solid phase primer (the solid phase strand) and another strand bound to a magnetic primer (the magnetic strand). Fourth, the two strands are separated from each other by applying a magnetic field, in which the solid phase is immobile and the magnetic strand is mobile.

Mian et al. used 5% glycerol and 5% ethylene glycol (column 16, lines 52-63) to facilitate the separation of the magnetic primer by reducing the melting temperature of the double stranded nucleic acid when a magnetic field is applied (column 11, lines 55-67; column 12, line 1-25). However, Mian et al. does not use the combination of glycerol and ethylene glycol at this concentration to denature of the double-stranded nucleic acids without applying a magnetic field for the purpose of cycle extension of DNA with high polymerization specificity. Magnetic cycle reaction applies a magnetic field to separate the two bound single-strands of nucleic acid. As would be recognized by someone having ordinary skill in this art, this is fundamentally different from the PCR technology that relies on melting the double stranded DNA by heat denaturation.

Given the serious deficiencies in each of Kurn and Mian, we submit that it is unreasonable to assume that a fair reading of both these references would have lead someone having ordinary skill in this art to our invention. It simply would not have been obvious to combine these references to achieve our claimed invention. Withdrawal of this rejection is therefore respectfully requested.

Claims 8-11 and 18-35 are rejected under 35 U.S.C. §103(a) as obvious over Hong et al. (U.S. Patent No. 6,165,765) in view of Mian et al. The Examiner has argued that it would have been obvious to modify the method of cycle primer extension at low

temperatures as taught by Hong with the teachings of Mian, as mentioned above regarding the previous rejection.

Regarding the Hong patent, we immediately note that all the citations referenced quoted by the Examiner (column 10, lines 42-57; column 15, lines 50-67; column 16, lines 1-16) are related to DNA sequencing without cycle amplification of primer—not cycle primer extension. These citations are concerned with the teachings of using a modified or unmodified DNA polymerase for ***a single, non-repetitive, non-cycle primer extension*** in the reaction mixture ***without glycerol added as a melting agent for the purpose of DNA sequencing***. A 5% and 50% of glycerol (column 23, lines 3-31) were used in the isolation and purification of DNA polymerase, not as a melting agent in an enzymatic reaction. This is in contrast to our claimed invention, which is directed to a method for extending an oligonucleotide primer or a pair of oligonucleotide primers via repeated cycles.

The Examiner cited column 8, lines 5-7 of the Hong patent, to support the assertion that Hong teaches a method for extending an oligonucleotide primer using an enzymatic cycle primer extension reaction at annealing temperatures between 45°C and 60°C and a melting temperature of 70°C. However, this is a misinterpretation of this passage, which refers to an optimum active temperature at 65° C for a thermostable DNA polymerase, not an annealing temperature between 45°C – 60°C or a melting temperature of 70°C.

As to the Examiner's reference to the '765 patent regarding the use of a DNA polymerase having homology (99-100%) to the instantly claimed SEQ ID Nos. 1-4, and the Examiner's citation of column 17, lines 1-23 regarding claim 29, we again emphasize that the polymerase referred to in the '765 patent is used for DNA sequencing with a single non-cycle extension of the molecules of a primer to different lengths in the presence of a chain terminator, namely a dye-labeled dideoxynucleotide. The same DNA polymerase may be used in our subject invention, but for different purpose in a different technology under different conditions.

The Examiner noted that Hong et al. fails to teach the use of a solution containing between about 10% and about 20% glycerol and/or ethylene glycol. As we mentioned

above, without the protection of glycerol, ethylene glycol or a mixture of glycerol and ethylene glycol between 10% and about 20%, the moderately thermostable Bacillus DNA polymerases are readily inactivated at 70° C. Again the secondary reference Mian does not make up for the primary reference's deficiencies. As mentioned above, Mian et al. does not use the combination of glycerol and ethylene glycol at this concentration to denature of the double-stranded nucleic acids without applying a magnetic field for the purpose of cycle extension of DNA with high polymerization specificity. Mian employs glycerol and ethylene glycol in a magnetic cycle reaction which, depending on applying a magnetic field to separate the two bound single-strands of nucleic acid, is fundamentally different from the PCR technology that relies on melting the double stranded DNA by heat denaturation.

We submit therefore that it would not have been obvious to one of ordinary skill in the art at the time our invention was made to combine the Hong and Main patents to reach our claimed invention. Reconsideration of this rejection is also requested.

In summary, then, someone having ordinary skill in this art, and having in hand any of Kurn, Mian, or Hong, would not have reasonably found our invention described by any combination of these references.

All of the Examiner's outstanding rejections and objections have been addressed, and the application is believed to be in allowable form. Notice to that effect is earnestly solicited.

If the Examiner has any questions or would like to make suggestions as to claim language, she is encouraged to contact Marlana K. Titus at (301) 977-7227. **[Please note that this is a new telephone number.]**

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